



## THE POTENTIATING ACTION OF ACETYLCHOLINE ON THAT OF ADRENALINE

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In the course of experiments on the stimulating action of acetylcholine on the heart (McDowall, 1946) it was observed that acetylcholine also sensitizes the organ to the action of a subsequent injection of adrenaline. The present investigation was carried out to determine how far the sensitizing action might be a general phenomenon. It was found that acetylcholine sensitizes the heart, the vessels, the blood pressure, the pupil and the intestine to the action of adrenaline.

### RESULTS

*Effect on the heart.* Hearts of rabbits and cats were perfused with Ringer-Locke's solution by the usual method (McDowall, 1946). Fig. 1 shows the potentiating effect of injecting acetylcholine on the response to adrenaline on the cat's heart. The result was the same for the rabbit's heart. In this, and in all subsequent experiments, adrenaline hydrochloride (Parke, Davis and Co.) was injected. It must be emphasized that a demonstration of the sensitizing effect of acetylcholine on the heart, as well as on other organs, cannot readily be repeated on the same preparation, as the increased sensitivity is often prolonged.

*Effect on the blood-vessels.* The method used was that described by Hemingway & McDowall (1926) in which the hind legs of a cat are perfused with Ringer's solution under a constant pressure, a record being taken of the change in the resistance to the flow, measured by a side-tube from the cannula entering the femoral artery. The drugs are injected through the rubber tubing leading to the cannula.

A dose of adrenaline, sufficient to cause a moderate constriction of the vessels, is injected and after the constriction has passed off, the vessels are dilated by means of acetylcholine (Fig. 2). Fig. 2 also shows the subsequent response to adrenaline; although, after the acetylcholine, the resistance is below that originally present, the rise caused by adrenaline is increased.

The sensitizing action on blood-vessels may also be seen if the volume of the venous outflow from the limbs is recorded in a chloralosed cat, in which coagulation of the blood is prevented by the injection of chlorazol fast pink.

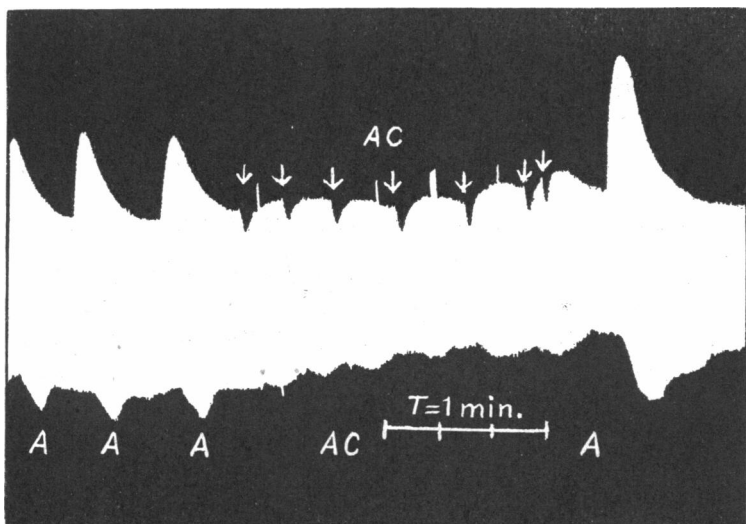


Fig. 1. Perfused cat's heart. Effect of seven injections of  $0.5 \mu\text{g.}$  acetylcholine (at arrows) in increasing the action of  $0.5 \mu\text{g.}$  adrenaline (at *A*). Time in min.

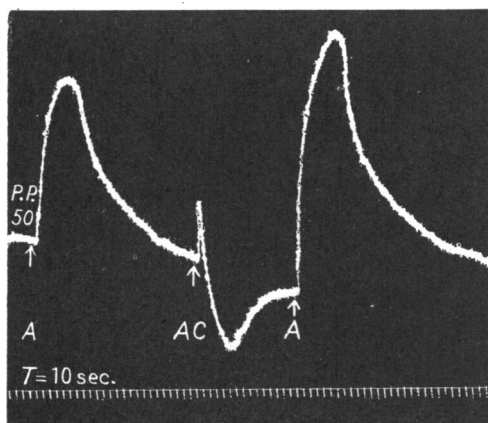


Fig. 2. Perfusion of blood-vessels of a cat's hind-limb. Record of perfusion pressure (P.P.). Effect of  $0.5 \mu\text{g.}$  of acetylcholine (at *AC*) on the constrictor response to  $0.25 \mu\text{g.}$  adrenaline (at *A*), P.P. = perfusion pressure, mm.  $\text{H}_2\text{O}$ . Time in 10 sec.

This sensitizing action on blood-vessels has already been reported by Danielopolu & Marcu (1939).

*Effect on the blood pressure.* This was studied on cats under chloralose anaesthesia. If the effects on the arterial blood pressure of intravenous injection of adrenaline before and after acetylcholine are compared, the results are varied. There may be an increased effect, no alteration or a reduced effect. The cause of the variation becomes apparent if simultaneous plethysmograph records are taken of corresponding skinned and unskinned limbs. The acetylcholine enhances both the vasodilator and vasoconstrictor effect of adrenaline, the former being best seen on the skinned limbs, where vessels of muscles predominate, and the latter in the normal limb during the first few minutes after the injection of acetylcholine, when the effect on the skin vessels predominates.

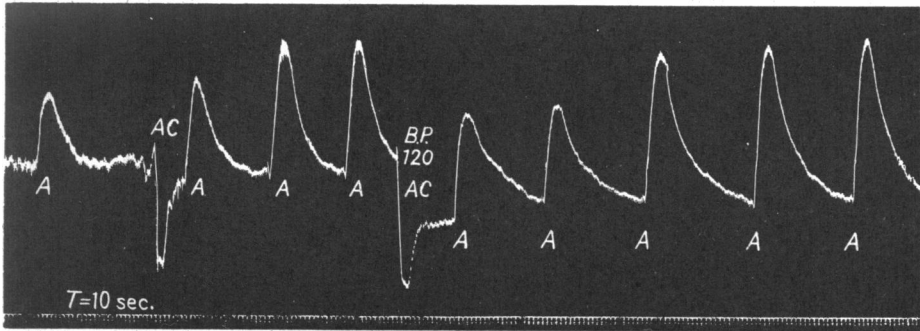


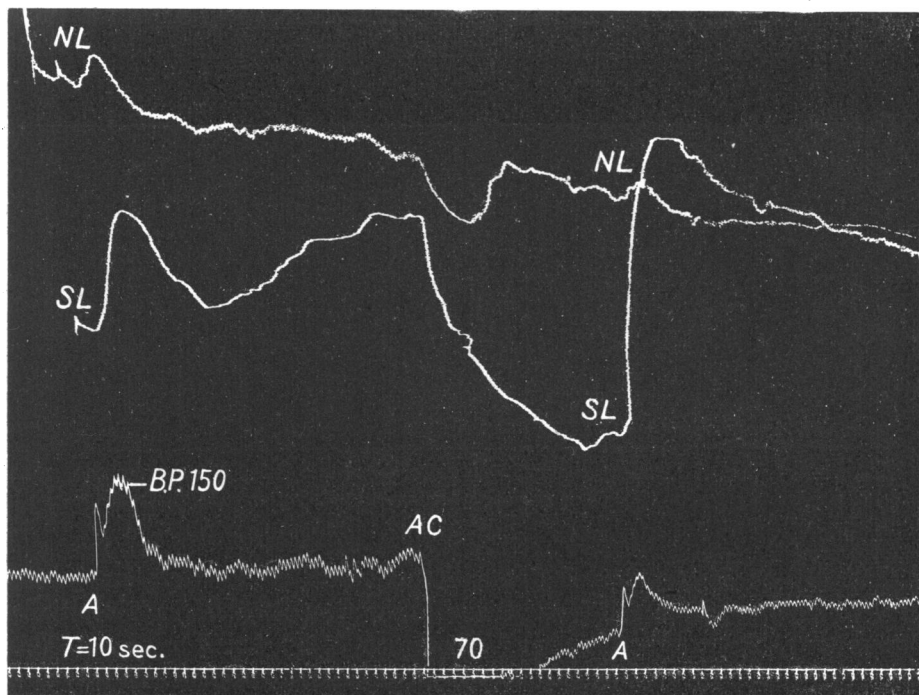
Fig. 3. Record of arterial blood pressure of a cat under chloralose. Effect of intravenous injection of  $10\text{ }\mu\text{g}$ . acetylcholine (at AC) on response to intravenous injection of  $5\text{ }\mu\text{g}$ . adrenaline (at A). Time in 10 sec.

In order to demonstrate the potentiating action of acetylcholine on the vasoconstrictor response, it is essential that the sympathetic, which is initially stimulated by chloralose (Vincent & Thompson, 1928) should be at rest. This state is reached by keeping the anaesthetized animal warm and undisturbed for about an hour, and is recognized by the fact that the pupils are constricted, though they still react to light and to sensory stimulation (McDowall, 1925). The record shown in Fig. 3 was obtained at this stage. At later stages in such experiments there is commonly a sustained constriction of the skin vessels, and in this condition the vasodilatation of muscle vessels by adrenaline predominates; this is now seen to be enhanced by the acetylcholine, with the result that the response of the blood pressure is reduced (Fig. 4). It may be noted that the dilatation of the vessels of the muscles is not a function of the rise of blood pressure.

*Effect on the pupil.* If a dose of about  $0.5\text{ }\mu\text{g}$ . of adrenaline is injected intravenously into a chloralosed cat, rested as above, the pupil is dilated very slightly. In this case, however, since the action of acetylcholine is so short-lived, owing to its rapid destruction in the blood, the sensitizing effect of

acetylcholine is not seen unless eserine is also injected at the same time to prevent the destruction. Subsequently, a similar dose of adrenaline may cause a full dilatation of the pupil.

*Effect on the isolated intestine.* The inhibitory action of adrenaline on the tone and movements of a piece of duodenum suspended in warm oxygenated Tyrode's solution is well known, and, with small doses, the effect is usually quite short-lived (Jendrassik, 1924). Generally, a repetition of the application of adrenaline to a fresh preparation results in a reduction of the response.



**Fig. 4.** Cat under chloralose. Effect of intravenous injection of  $50\mu\text{g.}$  of acetylcholine (at AC) on the response to an intravenous injection of  $5\mu\text{g.}$  adrenaline (at A). Upper record (N.L.) volume of normal limb in plethysmograph. Middle record (S.L.), volume of skinned limb in plethysmograph. Foot tied off. Lower record, arterial blood pressure. Time in 10 sec.

If a dose of adrenaline is used which is just sufficient to depress the intestine (the actual amount varies in different preparations) it is observed that if, after washing with fresh Tyrode's solution, a dose of acetylcholine is added to the bath, and the preparation again washed with fresh solution, the addition of a subsequent dose of adrenaline is often followed by an enhanced response. This may show itself either in a greater reduction of tone or in a more prolonged depression of the intestinal movements (Fig. 5). In some cases, however, the opposite result was obtained, and in spite of some fifty attempts, it has not been possible to determine the exact cause of this inconsistency.

More consistent results are obtained if a preparation is used which has been desensitized to adrenaline by several recent doses of adrenaline itself. If to such a preparation acetylcholine is added and washed off, the full sensitivity returns, and indeed the adrenaline may be more effective than originally (Fig. 6). Numerous attempts were made to demonstrate the sensitizing effect of the acetylcholine after atropine, but were unsuccessful because of the well-known variable effects of atropine itself on the intestine. In some cases atropine inhibits the intestine completely, and in others it appears to be ineffective.

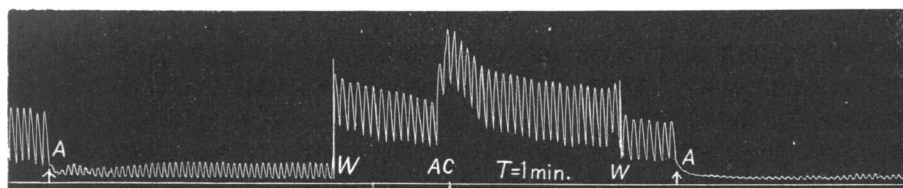


Fig. 5. Contractions of longitudinal muscle of isolated duodenum of rabbit suspended in Tyrode's solution. Potentiating effect of  $13 \mu\text{g.}$  of acetylcholine (at AC) on the response to  $6.5 \mu\text{g.}$  of adrenaline (at A). At W the bath was washed out and refilled with fresh Tyrode's solution.

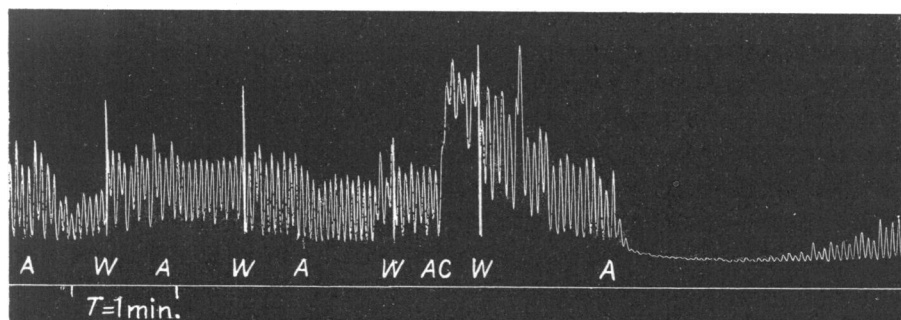


Fig. 6. Contractions of longitudinal muscle of isolated duodenum of rabbit suspended in Tyrode's solution. Sensitizing effect of  $0.1 \mu\text{g.}$  of acetylcholine (at AC) on response to  $1 \mu\text{g.}$  of adrenaline (at A). Muscle rendered insensitive to  $0.1 \mu\text{g.}$  adrenaline by repeated application. At W the bath was washed out and refilled with fresh Tyrode's solution.

#### DISCUSSION

Sensitization of tissues by acetylcholine to the action of adrenaline is widespread, and may be shown on the heart, blood-vessels, arterial blood pressure, pupil and intestine. The exact nature of the sensitization is not clear. If it is agreed that the acetylcholine stimulates chromaffin tissue which may liberate adrenaline, as suggested by Hoffmann, Hoffmann, Middleton & Talesnik (1945) in relation to the heart, then it must be assumed that such chromaffin tissue is much more widespread than is generally recognized. It is equally possible, however, that the sensitization is some direct effect on the contractile elements

of the heart. It is unlikely that the sensitization is due to stimulation of sympathetic ganglia, since the effect occurs also on the perfused vessels of the hind limbs, in which no ganglia have been described. The results generally are in accordance with a number of observations made by various workers on other parts of the body.

A synergic action of adrenaline and acetylcholine has already been described by Dale & Gaddum (1930) in relation to denervated muscle, by Bülbring (1945) in relation to the stimulation of the rectus abdominis of the frog, and by Bülbring & Burn (1942) in relation to transmission at the nerve-endings of a nerve-muscle preparation, while many clinical observers have emphasized the beneficial action of ephedrine in myasthenia gravis. A similar action of adrenaline and acetylcholine on the urinary bladder has been described by Mellanby & Pratt (1939, 1940). Such sensitization may have a considerable functional significance in relation to the parasympathetic system because adrenaline is secreted under conditions of emotional or physical stress at a time when parasympathetic action (i.e. the action of the vagus) is correspondingly reduced. The secretion of acetylcholine, however, just prior to the reduction may be looked upon as being partly responsible for the immediate increase of cardiac action, dilatation of the pupil and inhibition of the intestine produced by adrenaline. Thus the value of continuous liberation of acetylcholine by the parasympathetic system, which hitherto has appeared to be largely an extravagance of nature, becomes apparent.

The effect on the intestine is of special interest, as it suggests a new function for the abdominal vagus. It has always been difficult to understand why the action of adrenaline on the isolated intestine is so variable and often of such a short duration, while that *in vivo* is prolonged. In the isolated intestine the vagus has been severed and while, in some animals, there may remain some activity originating in the intestinal parasympathetic ganglia, as indicated by the action of atropine, such activity is not constant. This variability may merely reflect that of the vagus generally, which is known to be related to habitual activity. Thus it may be considered that animals of more active habit, which produce large amounts of acetylcholine at the vagus endings, are sensitized thereby to the action of adrenaline, and are thus made more efficient for exercise.

#### SUMMARY

Acetylcholine increases the response of the heart, blood-vessels, arterial blood pressure, pupil and intestine to adrenaline. The possible significance of the effect in relation to parasympathetic activity is discussed.

I should like to thank Dr M. L. Chakrabarty who, in the course of work in another connexion, repeated and confirmed the observations on the intestine.

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